United States Patent Application *20040005678*
Kind Code **A1**
Keasling, Jay ; et al. **January 8, 2004**

Biosynthesis of amorpha-4,11-diene

Abstract

Methods for synthesizing amorpha-4,11-diene synthase from isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. Amorpha-4,11-diene synthase is then produced using an optimized amorpha-4,11-diene synthase gene. The invention also provides nucleic acid sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

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Series Code: **10**
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U.S. Class at Publication: **435/146**; 435/193; 435/252.3; 435/320.1; 536/23.2
Intern'l Class: C12P 007/42; C12N 009/10; C07H 021/04; C12N 001/21; C12N 015/74

Claims

First Hit Fwd Refs

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L3: Entry 2 of 2 File: USPT Aug 8, 2000

US-PAT-NO: 6100451
DOCUMENT-IDENTIFIER: US 6100451 A
** See image for Certificate of Correction **

TITLE: Pathogen-inducible regulatory element

DATE-ISSUED: August 8, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chappell; Joseph	Lexington	KY		
Cornett; Catherine A. G.	Lexington	KY		
Yin; Shauhui	Lexington	KY		

US-CL-CURRENT: 800/298; 435/320.1, 435/419, 435/468, 536/24.1, 800/278, 800/279, 800/301, 800/317, 800/317.3, 800/319

CLAIMS:

What is claimed is:

1. A recombinant nucleic acid molecule comprising a pathogen- or elicitor-inducible transcriptional regulatory element comprising nucleotides 463-473 of SEQ ID NO:2, nucleotides 406 to 486 of SEQ ID NO:2, nucleotides 463 to 572 of SEQ ID NO:2, nucleotides 371 to 463 of SEQ ID NO:2, or nucleotides 411 to 457 of SEQ ID NO:2.

2. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a gene encoding a terpene cyclase.

3. The nucleic acid molecule of claim 2, wherein said terpene cyclase is a sesquiterpene cyclase.

4. The nucleic acid molecule of claim 3, wherein said transcriptional regulatory element directs expression of an epi-5-aristolochene synthase (EAS).

5. The nucleic acid molecule of claim 4, said nucleic acid molecule comprising the nucleotide sequence shown in FIG. 3A (SEQ ID NO:14) or a pathogen- or elicitor-inducible fragment thereof.

6. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule has the nucleotide sequence shown in FIG. 3A (SEQ ID NO: 14).

7. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a dicot.

8. The nucleic acid molecule of claim 7, wherein said dicot is a member of the Solanaceae.

9. The nucleic acid molecule of claim 8, wherein said Solanaceous plant is a member of the genus Nicotiana.

10. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a monocot.

11. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a gymnosperm.

12. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a conifer.

13. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is genomic DNA.

14. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is chemically-sythesized DNA.

15. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is a combination of genomic DNA and chemically-synthesized DNA.

16. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule is a combination of genomic DNA and cDNA or a combination of genomic DNA, cDNA, and chemically-synthesized DNA.

17. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element increases downstream gene expression in plant tissue in response to an elicitor or a plant pathogen.

18. The nucleic acid molecule of claim 17, wherein said plant pathogen is a fungus.

19. The nucleic acid molecule of claim 18, wherein said fungus is a member of the genus Phytophthora.

20. The nucleic acid molecule of claim 17, wherein said plant pathogen is a bacterium.

21. The nucleic acid molecule of claim 20, wherein said bacterium is a member of the genus Pseudomonas.

22. The nucleic acid molecule of claim 17, wherein said plant pathogen is a virus.

23. The nucleic acid molecule of claim 22, wherein said virus is tobacco mosaic virus.

24. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is induced by an elicitor.

25. The nucleic acid molecule of claim 17, wherein said elicitor is a fungal elicitor.

26. The nucleic acid molecule of claim 17, wherein said elicitor is a bacterial elicitor.

27. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule is operably linked to nucleotide sequences encoding a heterologous polypeptide.

28. The nucleic acid molecule of claim 27, wherein said heterologous polypeptide is capable of conferring disease-resistance to a plant.

29. The nucleic acid molecule of claim 28, wherein said heterologous polypeptide is an elicitin.

30. The nucleic acid molecule of claim 28, wherein said elicitin is a fungal elicitin.

31. The nucleic acid molecule of claim 30, said fungal elicitin being from Phytophthora.

32. The nucleic acid molecule of claim 31, said elicitin comprising a ParA1 polypeptide.

33. The nucleic acid molecule of claim 29, wherein said elicitin is a bacterial elicitin.

34. The nucleic acid molecule of claim 33, wherein said bacterial elicitin is a harpin.

35. The nucleic acid molecule of claim 27, wherein the expression of said heterologous polypeptide is mediated by one or more external agents.

36. The nucleic acid molecule of claim 27, wherein said nucleic acid molecule expresses said heterologous polypeptide in a cell-specific manner.

37. The nucleic acid molecule of claim 27, wherein said heterologous polypeptide is a pharmaceutical protein.

38. A vector comprising the DNA of claim 1.

39. The vector of claim 38, wherein said vector inducibly expresses a nucleotide sequence in a cell comprising said vector.

40. The vector of claim 39, said nucleotide sequence coding for a heterologous polypeptide.

41. A transgenic plant comprising the nucleic acid molecule of claim 1 integrated into the genome of said plant.

42. A transgenic plant comprising the nucleic acid molecule of claim 27 integrated into the genome of said plant.

43. A seed from the transgenic plant of claim 41.

44. A seed from the transgenic plant of claim 42.

45. A cell from the transgenic plant of claim 41.

46. A cell from the transgenic plant of claim 42.

47. A method of providing disease-resistance to a transgenic plant, said method comprising the steps of:

(a) producing a transgenic plant cell comprising the nucleic acid molecule of claim 28 integrated into the genome of said transgenic plant cell; and

(b) regenerating said transgenic plant from said plant cell wherein the expression of said nucleic acid molecule of claim 28 confers disease-resistance to said transgenic plant.

48. The method of claim 47, wherein said transgenic plant is a dicot.

49. The method of claim 48, wherein said dicot is a member of the Solanaceae.

50. The method of claim 49, wherein said member of the Solanaceae is a member of the genus Nicotiana.

51. The method of claim 47, wherein said transgenic plant is a monocot.

52. The method of claim 47, wherein said transgenic plant is a gymnosperm.

53. The method of claim 47, wherein said transgenic plant is a conifer.

54. A method of increasing the transcriptional expression of a downstream DNA sequence in a transgenic plant cell, said method comprising the steps of:

(a) producing a transgenic plant cell comprising the nucleic acid molecule of claim 1 positioned for increasing transcription of a downstream DNA sequence and integrated into the genome of said transgenic plant cell; and

(b) regenerating said transgenic plant from said plant cell.

WEST Search History

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DATE: Wednesday, June 09, 2004

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L3	sesquiterpene cyclase.clm.	2
☐	L2	sesquiterpene cyclase with dna	1
☐	L1	sesquiterpene cyclase	47

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=> s sesquiterpene cyclase and dna
L1          59 SESQUITERPENE CYCLASE AND DNA

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          40 DUP REM L1 (19 DUPLICATES REMOVED)

=> s l2 and 1990-1998/py
L3          19 L2 AND 1990-1998/PY

=> focus l3
PROCESSING COMPLETED FOR L3
L4          19 FOCUS L3 1-

=> s l4 and artemisia annua
L5           0 L4 AND ARTEMISIA ANNUA

=> d l4 1-19 ibib ab

L4   ANSWER 1 OF 19  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:        1995:487012  HCAPLUS
DOCUMENT NUMBER:         123:279727
TITLE:                   Cloning and bacterial expression of a
                         sesquiterpene cyclase from
                         Hyoscyamus muticus and its molecular comparison to
                         related terpene cyclases
AUTHOR(S):               Back, Kyoungwhan; Chappell, Joseph
CORPORATE SOURCE:        Agron. Dep., Univ. Kentucky, Lexington, KY,
                         40546-0091, USA
SOURCE:                  Journal of Biological Chemistry (1995),
                         270(13), 7375-81
                         CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER:               American Society for Biochemistry and Molecular
                         Biology
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   Genomic and cDNA clones for vetispiradiene synthase, sesquiterpene
     cyclase found in Hyoscyamus muticus, were isolated using a
     combination of reverse transcription-polymerase chain reactions and
     conventional cloning procedures.  RNA blot hybridization demonstrated an
     induction of mRNA consistent with the induction of cyclase enzyme activity
     in elicitor-treated cells, DNA blot hybridization indicated a
     gene family of 6 to 8 members, and bacterial expression of 3 cDNA clones
     indicated that each coded for a vetispiradiene synthase enzyme activity
     catalyzing the synthesis of a single reaction product.  Intron-exon
     organization of the vetispiradiene synthase gene was identical with that
     previously described for 5-epi-aristolochene synthase (tobacco
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sesquiterpene cyclase) and casbene synthase (castor bean diterpene cyclase), and the vetispiradiene synthase amino acid sequence was 77% identical with and 81% similar to the tobacco **sesquiterpene cyclase**. Regions of the vetispiradiene synthase sequence centered around amino acids 60, 100, and 370 were conspicuously different relative to the tobacco **sesquiterpene cyclase**. The sequence similarity between the tobacco and H. muticus enzymes is suggested to be reflective of the conservation of several partial reactions common to both enzymes, and the difference may be reflective of a partial reaction unique to each enzyme.

L4 ANSWER 2 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:710590 HCAPLUS
DOCUMENT NUMBER: 128:71516
TITLE: Regulation of **sesquiterpene cyclase** gene expression. Characterization of an elicitor- and pathogen-inducible promoter
AUTHOR(S): Yin, Shaohui; Mei, Leng; Newman, Jeffrey; Back, Kyoungwhan; Chappell, Joe
CORPORATE SOURCE: Plant Physiology/Biochemistry/Molecular Biology Program, University of Kentucky, Lexington, KY, 40546-0091, USA
SOURCE: Plant Physiology (**1997**), 115(2), 437-451
CODEN: PLPHAY; ISSN: 0032-0889
PUBLISHER: American Society of Plant Physiologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The promoter for a tobacco (Nicotiana tabacum) **sesquiterpene cyclase** gene, a key regulatory step in sesquiterpene phytoalexin biosynthesis, has been analyzed. The EAS4 promoter was fused to the .beta.-glucuronidase (GUS) reporter gene, and the temporal and spatial expression patterns of GUS activity were examd. in stably transformed plants and in transient expression assays using electroporated protoplasts of tobacco. No GUS activity was obsd. in any tissues under normal growth conditions. A low level of GUS activity was detected in wounded leaf, root, and stem tissues, whereas a much higher level was obsd. when these tissues were challenged with elicitors or microbial pathogens. The GUS expression pattern directed by the EAS4 promoter was identical to the induction patterns obsd. for the endogenous **sesquiterpene cyclase** genes. Neither exogenous salicylic acid nor Me jasmonate induced GUS expression; and H2O2 induced GUS expression to only a limited extent. Although the EAS4 promoter contains cis-sequences resembling previously identified transcriptional control motifs, other cis-sequences important for quant. and qual. gene expression were identified by deletion and gain-of-function analyses. The EAS4 promoter differs from previously described pathogen-/elicitor-inducible promoters because it only supports inducible gene expression and directs unique spatial expression patterns.
REFERENCE COUNT: 91 THERE ARE 91 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:532574 HCAPLUS
DOCUMENT NUMBER: 119:132574
TITLE: Gene family for an elicitor-induced **sesquiterpene cyclase** in tobacco
AUTHOR(S): Facchini, Peter J.; Chappell, Joseph
CORPORATE SOURCE: Agron. Dep., Univ. Kentucky, Lexington, KY, 40546-0091, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (**1992**), 89(22), 11088-92
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The initial step in the conversion of the isoprenoid intermediate farnesyl

diphosphate to the sesquiterpenoid phytoalexin capsidiol in elicitor-treated tobacco tissues is catalyzed by an inducible **sesquiterpene cyclase** [5-epi-aristolochene synthase (EAS)]. Two independent cDNA clones (cEAS1 and cEAS2) encoding EAS were isolated from an elicitor-induced tobacco cDNA library by differential hybridization and subsequently were characterized by hybrid selection-in vitro translation. Insertion of cEAS1, a partial cDNA clone encoding 175 C-terminal amino acids, into an Escherichia coli expression vector resulted in accumulation of a fusion protein immunodetectable with EAS-specific polyclonal antibodies. The cDNA clones were used to isolate two full-length EAS genes that mapped 5 kilobases (kb) apart on one 15-kb genomic clone. The nucleotide sequences of the structural gene components were identical from 388 base pairs (bp) upstream of the transcription initiation site to 40 bp downstream of the translation termination codon, suggesting a relatively recent duplication event. The genes consist of 1479-bp open reading frames, each contg. five introns and specifying 56,828-Da proteins. The N-terminal amino acid sequence deduced from the genomic clones was identical to the first 16 amino acids of the EAS protein identifiable by Edman degrdn. RNA blot hybridization with cEAS1 demonstrated a mRNA induction time course consistent with the induction of the EAS mRNA translational activity with max. levels 4-6 h after elicitation. EAS mRNA was not detected in control cells. **DNA** blot-hybridization anal. of genomic **DNA** revealed a copy no. of .apprxeq.12-15 for EAS-like genes in the tetraploid tobacco genome. The conservation of a putative allelic prenyl diphosphate binding motif is also discussed.

L4 ANSWER 4 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:717424 HCAPLUS
DOCUMENT NUMBER: 130:134851
TITLE: Characterization of the TAC box, a cis-element within an elicitor-inducible **sesquiterpene cyclase** promoter
AUTHOR(S): Newman, Jeffrey D.; Yin, Shaohui; Chappell, Joseph
CORPORATE SOURCE: Plant Physiology and Molecular Biology Program, University of Kentucky, Lexington, KY, 40546-0091, USA
SOURCE: Plant Journal (**1998**), 16(1), 1-12
CODEN: PLJUED; ISSN: 0960-7412
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The first unique step in the synthesis of the tobacco phytoalexin capsidiol is cyclization of farnesyl pyrophosphate catalyzed by 5-epi-aristolochene synthase (EAS), a **sesquiterpene cyclase**. Earlier work demonstrated that the elicitor-inducibility of this enzyme activity corresponded to the transcriptional activation of at least one gene, EAS4, of a rather complex gene family consisting of >10 members. To investigate the mechanism(s) controlling expression of this gene, fragments of the EAS4 promoter were examd. for binding by proteins in nuclear and whole-cell exts. A strong protein binding site (TAC box; ACTCTACAGTACTC) was identified between -245 and -232 by the electrophoretic mobility shift assay, and DNase I and methylation interference footprinting. Several distinctly migrating bands representing protein-TAC box complexes were also obsd. in the mobility shift assays, and the relative abundance of these bands varied in exts. from cells at different stages of EAS induction. The TAC box binding factor (TacBBF) was purified > 450-fold from crude whole-cell exts. by a combination of **DNA** affinity and cation exchange chromatog. The purified fractions were enriched for polypeptides of 17 and 19 kDa and the **DNA** binding properties of these prepns. were characterized. Mutation of 2 bp in the TAC box prevented protein binding in vitro and increased both basal and elicitor-inducible gene expression 2.5-fold in transgenic tobacco plants harboring promoter-GUS fusions, consistent with the notion that this cis-element functions as a silencer or repressor of EAS gene expression.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:547508 HCAPLUS
DOCUMENT NUMBER: 133:160583
TITLE: Plant regulatory elements involved in the hypersensitive response to infection and their uses
INVENTOR(S): Chappell, Joseph; Cornett, Catherine A. G.; Yin, Shauhui
PATENT ASSIGNEE(S): Board of Trustees of the University of Kentucky, USA
SOURCE: U.S., 39 pp., Cont.-in-part of U.S. Ser. No. 471,983, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
US 6100451	A	20000808	US 1995-577483	19951222	
US 5981843	A	19991109	US 1995-443639	19950518	
CA 2221348	AA	19961121	CA 1996-2221348	19960507	<--
WO 9636697	A1	19961121	WO 1996-US6452	19960507	<--
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI					
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML					
AU 9657321	A1	19961129	AU 1996-57321	19960507	<--
AU 724614	B2	20000928			
EP 828822	A1	19980318	EP 1996-915576	19960507	<--
R: BE, DE, FR, GB, IT					
CN 1191565	A	19980826	CN 1996-195657	19960507	<--
JP 11505423	T2	19990521	JP 1996-534883	19960507	
ES 2134170	A1	19990916	ES 1997-50002	19960507	
ES 2134170	B1	20001201			
SG 74110	A1	20000718	SG 1998-6010	19960507	
AP 924	A	20010104	AP 1997-1143	19960507	
W: KE, LS, MW, SD, SZ, UG					
ZA 9603957	A	19961125	ZA 1996-3957	19960517	<--
BR 9602338	A	19980113	BR 1996-2338	19960517	<--
TW 475945	B	20020211	TW 1996-85105864	19960517	
US 6605764	B1	20030812	US 1999-435380	19991105	

PRIORITY APPLN. INFO.:
US 1995-443639 A1 19950518
US 1995-471983 B2 19950606
US 1995-577483 A 19951222
WO 1996-US6452 W 19960507

AB Qual. transcriptional regulatory sequences functional in plants, plant tissue and in plant cells for inducible gene expression and quant. transcriptional regulatory sequences for increasing the transcriptional expression of downstream genetic information in plants, plant tissue and plant cells are disclosed. Also disclosed are methods and recombinant **DNA** mols. for improving the disease resistance of transgenic plants, esp. wherein an inducible promoter controls the expression of a protein capable of evoking the hypersensitive response in a plant. Regulatory elements derived from genes of phytoalexin biosynthesis that are induced as part of the hypersensitive response to infection by plant pathogenic fungi are described for use in increasing plant resistance to infection. The coding and regulatory regions of the tobacco epi-5-aristolochene synthase genes EAS3 and EAS4 genes of tobacco were cloned by std. methods. The promoter regions of these genes were used to drive expression of reporter genes in transgenic tobacco and the minimal

sequence requirements for elicitor induction of EAS4 expression detd. by deletion anal. Expression of the parA1 gene of Phytophthora parasitica from the EAS4 promoter in transgenic plants led to increased resistance to infection by P. parasitica var. Nicotianae.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1991:529134 BIOSIS
DOCUMENT NUMBER: PREV199192140594; BA92:140594
TITLE: EXPRESSION OF A FUNGAL **SESQUITERPENE CYCLASE** GENE IN TRANSGENIC TOBACCO.
AUTHOR(S): HOHN T M [Reprint author]; OHLROGGE J B
CORPORATE SOURCE: MYCOTOXIN RES UNIT, NATL CENT AGRIC UTILIZATION RES, AGRIC RES SERVICE, US DEP AGRIC, PEORIA, ILL 61604, USA
SOURCE: Plant Physiology (Rockville), (1991) Vol. 97, No. 1, pp. 460-462.
CODEN: PLPHAY. ISSN: 0032-0889.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 19 Nov 1991
Last Updated on STN: 8 Jan 1992

AB The complete coding sequence for the trichodiene synthase gene from Fusarium sporotrichioides was introduced into tobacco (Nicotiana tabacum) under the regulation of the cauliflower mosiac virus 35S promoter. Expression of trichodiene synthase was demonstrated in the leaves of transformed plants. Leaf homogenates incubated with [3H]farnesyl pyrophosphate produced trichodiene as a major product. Trichodiene was detected in the leaves of a transformed plant at a level of 5 to 10 nanograms per gram fresh weight. The introduction of a fungal **sesquiterpene cyclase** gene into tobacco has resulted in the expression of an active enzyme and the accumulation of low levels of its sesquiterpenoid product.

L4 ANSWER 7 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:227090 HCAPLUS
DOCUMENT NUMBER: 118:227090
TITLE: Aristolochene synthase. Isolation, characterization, and bacterial expression of a sesquiterpenoid biosynthetic gene (Ari1) from Penicillium roqueforti
AUTHOR(S): Proctor, Robert H.; Hohn, Thomas M.
CORPORATE SOURCE: Mycotoxin Res. Unit, U. S. Dep. Agric., Peoria, IL, 61604, USA
SOURCE: Journal of Biological Chemistry (**1993**), 268(6), 4543-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Aristolochene is the likely precursor of the sesquiterpenoid toxins produced by a no. of filamentous fungi. One of these, PR-toxin, is produced by Penicillium roqueforti. Here the isolation of a gene (Ari1) coding for the **sesquiterpene cyclase**, aristolochene synthase (AS), from P. roqueforti is reported. Nucleotide sequence anal. of genomic and cDNA clones revealed that the Ari1 gene contains 2 introns. A Protein A/AS fusion enzyme was expressed in Escherichia coli and shown to have **sesquiterpene cyclase** activity. Anal. of the Protein A/AS fusion enzyme reaction mixts. by TLC and gas chromatog./mass spectrometry identified aristolochene as a major product. The Ari1 gene encodes a polypeptide of mol. wt. 39,200. Expression of Ari1 occurs in stationary phase cultures of P. roqueforti and appears to be transcriptionally regulated.

L4 ANSWER 8 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:58175 HCAPLUS

DOCUMENT NUMBER: 118:58175
TITLE: Overproduction of soluble trichodiene synthase from Fusarium sporotrichioides in Escherichia coli
AUTHOR(S): Cane, David E.; Wu, Zhen; Oliver, John S.; Hohn, Thomas M.
CORPORATE SOURCE: Dep. Chem., Brown Univ., Providence, RI, 02912, USA
SOURCE: Archives of Biochemistry and Biophysics (**1993**), 300(1), 416-22
CODEN: ABBIA4; ISSN: 0003-9861
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Trichodiene synthase is a **sesquiterpene cyclase** isolated from various fungal spp. that catalyzes the cyclization of farnesyl diphosphate (FPP) to trichodiene. The trichodiene synthase gene (Tox5) of F. sporotrichioides has previously been cloned and expressed as 0.05-0.1% of total cell protein in E. coli. Polymerase chain reaction was used to amplify the trichodiene coding sequence carried on the plasmid pTS56-1. The resulting **DNA**, carrying a BamHI restriction site and the T7 gene 10 ribosome binding site and translational spacer element immediately upstream of the ATG start codon as well as a HindIII site adjacent to the translational stop codon, was inserted into the corresponding sites of the expression vector pLM1. The latter vector carried the promoter and translational leader sequence from T7 gene 10 and the E. coli rmBT1T2 tandem transcription terminator. This construct was cloned into E. coli BL21(DE3). The resulting transformants, when induced with iso-Pr .beta.-D-thiogalactoside, produced trichodiene synthase as 20-30% of total sol. protein. The recombinant synthase, which could be purified 5-fold to homogeneity by (NH4)2SO4 pptn., ion-exchange chromatog. on Q Sepharose, and gel filtration on Superose 12, was identical to native protein in steady-state kinetic parameters and mobility on SDS-PAGE and had the expected MENFP N-terminal sequence.

L4 ANSWER 9 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:759657 HCAPLUS
DOCUMENT NUMBER: 123:220086
TITLE: Accurate in vitro transcription from circularized plasmid templates by plant whole cell extracts
AUTHOR(S): Zhu, Qun; Chappell, Joseph; Hedrick, Susan A.; Lamb, Chris
CORPORATE SOURCE: Plant Biology Laboratory, Salk Institute Biological Studies, La Jolla, CA, 92037, USA
SOURCE: Plant Journal (**1995**), 7(6), 1021-30
CODEN: PLJUED; ISSN: 0960-7412
PUBLISHER: Blackwell
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A convenient in vitro transcription system using monocot and dicot whole cell exts. and circular **DNA** templates has been developed. The system consists of incubating template and whole cell ext. to generate initiation complexes, followed by addn. of nucleotide triphosphates to support elongation, and primer extension assay to detect authentic transcripts. This in vitro transcription system required circularized templates and was essentially inactive with linearized templates. Accurate in vitro transcription of a rice phenylalanine ammonia-lyase (PAL) promoter-.beta.-glucuronidase (GUS) gene fusion and a tobacco **sesquiterpene cyclase** promoter-GUS gene fusion was examd. in their homologous whole cell exts., and the optimal concns. for several reaction components, including **DNA** template, whole cell ext., monovalent and divalent cations, were detd. for specific initiation from the in vivo start site. Transcription was inhibited by low concns. of .alpha.-amanitin, demonstrating that the reaction was mediated by RNA polymerase II. Accurate transcription initiation was dependent on the TATA-box motif within the resp. promoters. Based on the effect of delayed addn. of sarkosyl at a concn. sufficient to inhibit transcription initiation but not elongation, three to four rounds of transcription were

initiated in std. assays.

L4 ANSWER 10 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:131950 BIOSIS
DOCUMENT NUMBER: PREV200300131950
TITLE: Induction of a unique **sesquiterpene cyclase** by secondary signals released from pathogen-challenged cells.
AUTHOR(S): Schoenbeck, Mark A. [Reprint Author]; Lusso, Marcos [Reprint Author]; Chappell, Joe [Reprint Author]; Mandujano-Chavez, Alejandra
CORPORATE SOURCE: Plant Physiology/Biochemistry/Molecular Biology Program, University of Kentucky, Lexington, KY, USA
SOURCE: Plant Biology (Rockville), (1998) Vol. 1998, pp. 152. print.
Meeting Info.: Annual Meeting of the American Society of Plant Physiologists combined with the 9th International Conference on Arabidopsis Research. Madison, WI, USA. June 27-July 01, 1998. American Society of Plant Physiologists (ASPP).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Mar 2003
Last Updated on STN: 12 Mar 2003

L4 ANSWER 11 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:14554 BIOSIS
DOCUMENT NUMBER: PREV199698586689
TITLE: Characterization of potato **sesquiterpene cyclase** cDNA clones.
AUTHOR(S): Zook, M.
CORPORATE SOURCE: Dep. Bot. Plant Pathol., Mich. State Univ., East Lansing, MI 48824, USA
SOURCE: Phytopathology, (1995) Vol. 85, No. 10, pp. 1161.
Meeting Info.: Annual Meeting of the American Phytopathological Association. Pittsburgh, Pennsylvania, USA. August 12-16, 1995.
CODEN: PHYTAJ. ISSN: 0031-949X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Jan 1996
Last Updated on STN: 4 Jan 1996

L4 ANSWER 12 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1994:142694 BIOSIS
DOCUMENT NUMBER: PREV199497155694
TITLE: Partial genomic sequence of potato **sesquiterpene cyclase**.
AUTHOR(S): Zook, M.
CORPORATE SOURCE: Dep. Botany and Plant Pathol., Michigan State Univ., East Lansing, MI 48824, USA
SOURCE: Phytopathology, (1993) Vol. 83, No. 12, pp. 1382.
Meeting Info.: Joint Meeting of the American Phytopathological Society and the Society of Nematologists on Plant Pathology Beyond 2000. Nashville, Tennessee, USA. November 6-10, 1993.
CODEN: PHYTAJ. ISSN: 0031-949X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Mar 1994
Last Updated on STN: 31 Mar 1994

L4 ANSWER 13 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1992:405136 BIOSIS
DOCUMENT NUMBER: PREV199243061011; BR43:61011
TITLE: ISOLATION AND CHARACTERIZATION OF CDNA AND GENOMIC CLONES ENCODING AN INDUCIBLE **SESQUITERPENE CYCLASE** FROM TOBACCO.
AUTHOR(S): FACCHINI P J [Reprint author]; CHAPPELL J
CORPORATE SOURCE: PLANT PHYSIOLOGY/BIOCHEMISTRY/MOLECULAR BIOLOGY PROGRAM, AGRONOMY DEP, UNIVERSITY KENTUCKY, LEXINGTON, KY 40546, USA
SOURCE: Plant Physiology (Rockville), (1992) Vol. 99, No. 1 SUPPL, pp. 86.
Meeting Info.: ANNUAL MEETING OF THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, PITTSBURGH, PENNSYLVANIA, USA, AUGUST 1-5, 1992. PLANT PHYSIOL (BETHESDA).
CODEN: PLPHAY. ISSN: 0032-0889.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 26 Aug 1992
Last Updated on STN: 1 Oct 1992

L4 ANSWER 14 OF 19 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN
ACCESSION NUMBER: 1999:22259 AGRICOLA
DOCUMENT NUMBER: IND21971988
TITLE: Cloning and bacterial expression of **sesquiterpene cyclase**, a key branch point enzyme for the synthesis of sesquiterpenoid phytoalexin capsidiol in UV-challenged leaves of Capsicum annuum.
AUTHOR(S): Back, K.; He, S.; Kim, K.U.; Shin, D.H.
CORPORATE SOURCE: Chonnam National University, Kwangju, South Korea.
AVAILABILITY: DNAL (450 P699)
SOURCE: Plant and cell physiology, **Sept 1998.** Vol. 39, No. 9. p. 899-904
Publisher: Kyoto, Japan : Japanese Society of Plant Physiologists.
CODEN: PCPHA5; ISSN: 0032-0781
NOTE: Includes references
PUB. COUNTRY: Japan
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English
AB **Sesquiterpene cyclase**, a branch point enzyme in the general isoprenoid pathway for the synthesis of phytoalexin capsidiol, was induced in detached leaves of Capsicum annuum (pepper) by UV treatment. The inducibility of cyclase enzyme activities paralleled the absolute amount of cyclase protein(s) of pepper immunodetected by monoclonal antibodies raised against tobacco **sesquiterpene cyclase** . A cDNA library was constructed with poly(A)+ RNA isolated from 24 h UV-challenged leaves of pepper. A cDNA clone for **sesquiterpene cyclase** in pepper was isolated by using a tobacco 5-epi aristolochene synthase gene as a heterologous probe. The predicted protein encoded by this cDNA was comprised of 559 amino acids and had a relative molecular mass of 65,095. The primary structural information from the cDNA clone revealed that it shared 77%, 72% and 49% identity with 5-epi aristolochene, vetispiradiene, and cadinene synthase, respectively. The enzymatic product catalyzed by the cDNA clone in bacteria was identified as 5-epi aristolochene, as judged by argentation TLC. RNA blot hybridization demonstrated the induction of an mRNA consistent with the induction of cyclase enzyme activity in UV-treated pepper.

L4 ANSWER 15 OF 19 AGRICOLA Compiled and distributed by the National

ACCESSION NUMBER: 1998:49601 AGRICOLA
DOCUMENT NUMBER: IND21378981
TITLE: **Sesquiterpene cyclase** is not a determining factor for elicitor- and pathogen-induced capsidiol accumulation in tobacco.
AUTHOR(S): Keller, H.; Czernic, P.; Ponchet, M.; Ducrot, P.H.; Back, K.; Chappell, J.; Ricci, P.; Marco, Y.
AVAILABILITY: DNAL (450 P693)
SOURCE: Planta, **July 1998.** Vol. 205, No. 3. p. 467-476
Publisher: Berlin ; New York : Springer-Verlag, 1925-
CODEN: PLANAB; ISSN: 0032-0935
NOTE: Includes references
PUB. COUNTRY: Germany
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English

AB The induction of **sesquiterpene cyclase**, a key phytoalexin biosynthetic enzyme, and the accumulation of phytoalexins in relation to the induction of a hypersensitive response (HR) and cell necrosis in tobacco (Nicotiana tabacum L.) were investigated. When tobacco leaves were inoculated with virulent or avirulent isolates of Ralstonia solanacearum, steady-state levels of mRNA complementary to cDNA of the sensitivity-related (sts) gene str319 were dramatically induced. This cDNA clone is greater than 90% homologous with a gene coding for 5-epi-aristolochene synthase (EAS), previously described as a branch-point enzyme regulating the synthesis of capsidiol, the major sesquiterpenoid phytoalexin found in tobacco. Accumulation of EAS transcripts in leaves after inoculation with virulent and avirulent strains of R. solanacearum, or after treatment with necrotizing or non-necrotizing elicitins was rapid but transient, and restricted to the site of infiltration. Two highly similar **sesquiterpene cyclase** activities, 5-epi-aristolochene synthase and a vetispiradiene synthase-like activity, were found in extracts of elicitin-challenged and R. solanacearum-inoculated tobacco. Under all conditions tested, the induction of cyclase activity was closely correlated with induction of the cyclase mRNA level. In contrast, high levels of capsidiol were found only after treatment with the necrosis-inducing elicitin cryptogein, or after infiltration with HR-inducing bacterial strains. Low levels of capsidiol did accumulate after application of capsicein, an elicitin that induces little or no necrosis on tobacco, or after infection with a virulent bacterium. Hence, capsidiol accumulation, not 5-epi-aristolochene synthase gene expression or total **sesquiterpene cyclase** enzyme activity, appears to be a good marker for the HR of tobacco.

L4 ANSWER 16 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:232423 BIOSIS
DOCUMENT NUMBER: PREV199698796552
TITLE: A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of taxol biosynthesis.
AUTHOR(S): Wildung, Mark R.; Croteau, Rodney [Reprint author]
CORPORATE SOURCE: Inst. Biol. Chem., Washington State University, Pullman, WA 99164-6340, USA
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 16, pp. 9201-9204.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
OTHER SOURCE: EMBL-U48796; Genbank-U48796
ENTRY DATE: Entered STN: 28 May 1996
Last Updated on STN: 11 Jul 1996

AB The committed step of taxol (paclitaxel) biosynthesis is catalyzed by

taxa-4(5),11(12)-diene synthase, a diterpene cyclase responsible for transforming the ubiquitous isoprenoid intermediate geranylgeranyl diphosphate to the parent olefin with a taxane skeleton. T obtain the corresponding cDNA clone, a set of degenerate primers was constructed based on consensus sequences of related monoterpene, sesquiterpene, an diterpene cyclases. Two of these primers amplified 83-base pair fragment that was cyclase-like in sequence and that was employed as a hybridization probe to screen a cDNA library constructed from poly(A)+ RNA extracted from Pacific yew (Taxus brevifolia) stems. Twelve independent clones with insert size in excess of 2 kilobase pairs were isolated and partially sequenced. One of these cDNA isolates was functionally expressed in Escherichia coli, yielding a protein that was catalytically active in converting geranylgeranyl diphosphate to a diterpene olefin that was confirmed to be taxa4(5),11(12)-diene by combined capillary gas chromatography-mass spectrometry. The sequence specifies an open reading frame of 2586 nucleotides, and the complete deduced polypeptide, including a long presumptive plastidial targeting peptide, contains 862 amino acid residues and has a molecular weight of 98,303, compared with about 79,000 previously determined for the mature native enzyme. Sequence comparisons with monoterpene, sesquiterpene, and diterpene cyclases of plant origin indicate a significant degree of similarity between these enzymes; the taxadiene synthase most closely resembles (46% identity, 67% similarity) abietadiene synthase, a diterpene cyclase from grand fir.

L4 ANSWER 17 OF 19 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN

ACCESSION NUMBER: 96:53030 AGRICOLA
DOCUMENT NUMBER: IND20530195
TITLE: Identifying functional domains within terpene cyclases using a domain-swapping strategy.
AUTHOR(S): Back, K.; Chappell, J.
CORPORATE SOURCE: University of Kentucky, Lexington, KY.
AVAILABILITY: DNAL (500 N21P)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, **June 25, 1996.** Vol. 93, No. 13. p. 6841-6845
Publisher: Washington, D.C. : National Academy of Sciences,
CODEN: PNASA6; ISSN: 0027-8424
NOTE: Includes references
PUB. COUNTRY: District of Columbia; United States
DOCUMENT TYPE: Article; Conference
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB Cyclic terpenes and terpenoids are found throughout nature. They comprise an especially important class of compounds from plants that mediate plant-environment interactions, and they serve as pharmaceutical agents with antimicrobial and anti-tumor activities. Molecular comparisons of several terpene cyclases, the key enzymes responsible for the multistep cyclization of C10, C15, and C20 allylic diphosphate substrates, have revealed a striking level of sequence similarity and conservation of exon position and size within the genes. Functional domains responsible for a terminal enzymatic step were identified by swapping regions approximating exons between a Nicotiana tabacum 5-epiaristolochene synthase (TEAS) gene and a Hyoscyamus muticus vetispiradiene synthase (HVS) gene and by characterization of the resulting chimeric enzymes expressed in bacteria. While exon 4 of the TEAS gene conferred specificity for the predominant reaction products of the tobacco enzyme, exon 6 of the HVS gene conferred specificity for the predominant reaction products) of the Hyoscyamus enzyme. Combining these two functional domains of the TEAS and HVS genes resulted in a novel enzyme capable of synthesizing reaction products reflective of both parent enzymes. The relative ratio of the TEAS and HVS reaction products was also influenced by the source of exon 5 present in

the new chimeric enzymes. The association of catalytic activities with conserved but separate exonic domains suggests a general means for generating additional novel terpene cyclases.

L4 ANSWER 18 OF 19 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN

ACCESSION NUMBER: 1999:8743 AGRICOLA
DOCUMENT NUMBER: IND21961068
TITLE: Germacrene C synthase from Lycopersicon esculentum cv. VFNT Cherry tomato: cDNA isolation, characterization, and bacterial expression of the multiple product **sesquiterpene cyclase**.
AUTHOR(S): Colby, S.M.; Crock, J.; Dowdle-Rizzo, B.; Lemaux, P.G.; Croteau, R.
CORPORATE SOURCE: University of California, Berkeley, CA.
AVAILABILITY: DNAL (500 N21P)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, **Mar 3, 1998.** Vol. 95, No. 5. p. 2216-2221
Publisher: Washington, D.C. : National Academy of Sciences,
CODEN: PNASA6; ISSN: 0027-8424
NOTE: Includes references
PUB. COUNTRY: District of Columbia; United States
DOCUMENT TYPE: Article; Conference
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB Germacrene C was found by GC-MS and NMR analysis to be the most abundant sesquiterpene in the leaf oil of Lycopersicon esculentum cv. VFNT Cherry, with lesser amounts of germacrene A, guaia-6,9-diene, germacrene B, beta-caryophyllene, alpha-humulene, and germacrene D. Soluble enzyme preparations from leaves catalyzed the divalent metal ion-dependent cyclization of [1-3H]farnesyl diphosphate to these same sesquiterpene olefins, as determined by radio-GC. To obtain a germacrene synthase cDNA, a set of degenerate primers was constructed based on conserved amino acid sequences of related terpenoid cyclases. With cDNA prepared from leaf epidermis-enriched mRNA, these primers amplified a 767-bp fragment that was used as a hybridization probe to screen the cDNA library. Thirty-one clones were evaluated for functional expression of terpenoid cyclase activity in Escherichia coli by using labeled geranyl, farnesyl, and geranylgeranyl diphosphates as substrates. Nine cDNA isolates expressed sesquiterpene synthase activity, and GC-MS analysis of the products identified germacrene C with smaller amounts of germacrene A, B, and D. None of the expressed proteins was active with geranylgeranyl diphosphate; however, one truncated protein converted geranyl diphosphate to the monoterpene limonene. The cDNA inserts specify a deduced polypeptide of 548 amino acids (M(r) = 64,114), and sequence comparison with other plant sesquiterpene cyclases indicates that germacrene C synthase most closely resembles cotton delta-cadinene synthase (50% identity).

L4 ANSWER 19 OF 19 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN

ACCESSION NUMBER: 94:24633 AGRICOLA
DOCUMENT NUMBER: IND20380755
TITLE: 4S-limonene synthase from the oil glands of spearmint (Mentha spicata): cDNA isolation, characterization, and bacterial expression of the catalytically active monoterpene cyclase.
AUTHOR(S): Colby, S.M.; Alonso, W.R.; Katahira, E.J.; McGarvey, D.J.; Croteau, R.
AVAILABILITY: DNAL (381 J824)

SOURCE: The Journal of biological chemistry, **Nov 5, 1993.** Vol. 268, No. 31. p. 23016-23024
Publisher: Baltimore, Md. : American Society for Biochemistry and Molecular Biology.
CODEN: JBCHA3; ISSN: 0021-9258
NOTE: Includes references
PUB. COUNTRY: Maryland; United States
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB The committed step in the biosynthesis of monoterpenes in mint (Mentha) species is the cyclization of geranyl pyrophosphate to the olefin (-)-4S-limonene catalyzed by limonene synthase (cyclase). Internal amino acid sequences of the purified enzyme from spearmint oil glands were utilized to design three distinct oligonucleotide probes. These probes were subsequently employed to screen a spearmint leaf cDNA library, and four clones were isolated. Three of these cDNA isolates were full-length and were functionally expressed in Escherichia coli, yielding a peptide that is immunologically recognized by polyclonal antibodies raised against the purified limonene synthase from spearmint and that is catalytically active in generating from geranyl pyrophosphate a product distribution identical to that of the native enzyme (principally limonene with small amounts of the coproducts alpha- and beta-pinene and myrcene). The longest open reading frame is 1800 nucleotides and the deduced amino acid sequence contains a putative plastidial transit peptide of approximately 90 amino acids and a mature protein of about 510 residues corresponding to the native enzyme. Several nucleotide differences in the 5'-untranslated region of all three full-length clones suggest the presence of several limonene synthase genes and/or alleles in the allotetraploid spearmint genome. Sequence comparisons with a **sesquiterpene cyclase**, epi-aristolochene synthase from tobacco, and a diterpene cyclase, casbene synthase from castor bean, demonstrated a significant degree of similarity between these three terpenoid cyclase types, the first three examples of this large family of catalysts to be described from higher plants.

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=> s (amorpha-4,11-diene synthase or amorphadiene synthase) and (dna or rna or nucleic acid
   1 FILES SEARCHED...
L6            9 (AMORPHA-4,11-DIENE SYNTHASE OR AMORPHADIENE SYNTHASE) AND (DNA
                OR RNA OR NUCLEIC ACID)

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7            6 DUP REM L6 (3 DUPLICATES REMOVED)

=> d l7 1-6 ibib ab

L7   ANSWER 1 OF 6  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2004:18837  HCAPLUS
DOCUMENT NUMBER:          140:92683
TITLE:                    Preparation of amorpha-4,11-diene with transgenic
                          microorganisms producing isopentenyl- and
                          dimethylallyl pyrophosphates
INVENTOR(S):              Keasling, Jay; Martin, Vincent; Pitera, Douglas;
                          Withers, Sydnor T.; Newman, Jack
PATENT ASSIGNEE(S):       USA
SOURCE:                   U.S. Pat. Appl. Publ., 75 pp., Cont.-in-part of U.S.
                          Ser. No. 6,909.
                          CODEN: USXXCO
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   2
PATENT INFORMATION:
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PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004005678	A1	20040108	US 2003-411066	20030409
US 2003148479	A1	20030807	US 2001-6909	20011206

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206

AB Methods for synthesizing amorpha-4,11-diene from isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous **nucleic acid** sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. Amorpha-4,11-diene is then produced with the transgenic microorganism which is further transformed with an optimized **amorpha-4,11-diene synthase** gene. The amorpha-4,11-diene may be used in synthesis of the antimalarial drug artemisinin. Thus, amorpha-4,11-diene was prepd. from mevalonate supplied in the medium with Escherichia coli transformed with plasmid pBBRMDIS-2, contg. the yeast genes idi (for isopentenyl pyrophosphate isomerase) and ispA (for farnesyl pyrophosphate synthase) and the genes for mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, and **amorpha-4,11-diene synthase**. The yield was 2 .mu.g amorpha-4,11-diene/mL.

L7 ANSWER 2 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:609986 HCAPLUS
DOCUMENT NUMBER: 139:160786
TITLE: Biosynthesis of isopentenyl pyrophosphate using recombinant microbial metabolic pathways
INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas; Kim, Seon-Won; Withers, Sydnor T.; Yoshikuni, Yasuo; Newman, Jack; Khlebnikov, Artem Valentinovich
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 40 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003148479	A1	20030807	US 2001-6909	20011206
US 2004005678	A1	20040108	US 2003-411066	20030409

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206

AB Methods for synthesizing isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous **nucleic acid** sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. A related method comprises introducing into a host microorganism an intermediate in the mevalonate pathway and at least one heterologous **nucleic acid** sequence, each sequence coding for an enzyme in the mevalonate pathway necessary for converting the intermediate into isopentenyl pyrophosphate. The invention also provides **nucleic acid** sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

L7 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 2003-18363 BIOTECHDS
TITLE: Engineering a mevalonate pathway in Escherichia coli for production of terpenoids;
vector-mediated gene transfer and expression in host cell for strain improvement and terpene preparation
AUTHOR: MARTIN VJJ; PITERA DJ; WITHERS ST; NEWMAN JD; KEASLING JD
CORPORATE SOURCE: Univ Calif Berkeley; Lawrence Berkeley Natl Lab
LOCATION: Keasling JD, Univ Calif Berkeley, Dept Chem Engn, 201 Gilman Hall, Berkeley, CA 94720 USA

SOURCE: NATURE BIOTECHNOLOGY; (2003) 21, 7, 796-802
ISSN: 1087-0156
DOCUMENT TYPE: Journal
LANGUAGE: English
AB AUTHOR ABSTRACT - Isoprenoids are the most numerous and structurally diverse family of natural products. Terpenoids, a class of isoprenoids often isolated from plants, are used as commercial flavor and fragrance compounds and antimalarial or anticancer drugs. Because plant tissue extractions typically yield low terpenoid concentrations, we sought an alternative method to produce high-value terpenoid compounds, such as the antimalarial drug artemisinin, in a microbial host. We engineered the expression of a synthetic amorpha- 4,11-diene synthase gene and the mevalonate isoprenoid pathway from Saccharomyces cerevisiae in Escherichia coli. Concentrations of amorphadiene, the sesquiterpene olefin precursor to artemisinin, reached 24 mug caryophyllene equivalent/ml. Because isopentenyl and dimethylallyl pyrophosphates are the universal precursors to all isoprenoids, the strains developed in this study can serve as platform hosts for the production of any terpenoid compound for which a terpene synthase gene is available. (7 pages)

L7 ANSWER 4 OF 6 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN DUPLICATE 1
ACCESSION NUMBER: 2001:63594 AGRICOLA
DOCUMENT NUMBER: IND23222235
TITLE: **Amorpha-4,11-diene synthase**: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin.
AUTHOR(S): Wallaart, T.E.; Bouwmeester, H.J.; Hille, J.; Poppinga, L.; Maijers, N.C.A.
AVAILABILITY: DNAL (450 P693)
SOURCE: Planta, Feb 2001. Vol. 212, No. 3. p. 460-465
Publisher: Berlin ; New York : Springer-Verlag, 1925-
CODEN: PLANAB; ISSN: 0032-0935
NOTE: Includes references
PUB. COUNTRY: Germany
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English
AB The sesquiterpenoid artemisinin, isolated from the plant Artemisia annua L., and its semi-synthetic derivatives are a new and very effective group of antimalarial drugs. A branch point in the biosynthesis of this compound is the cyclisation of the ubiquitous precursor farnesyl diphosphate into the first specific precursor of artemisinin, namely amorpha-4,11-diene. Here we describe the isolation of a cDNA clone encoding **amorpha-4,11-diene synthase**. The deduced amino acid sequence exhibits the highest identity (50%) with a putative sesquiterpene cyclase of A. annua. When expressed in Escherichia coli, the recombinant enzyme catalyses the formation of amorpha-4,11-diene from farnesyl diphosphate. Introduction of the gene into tobacco (Nicotiana tabacum L.) resulted in the expression of an active enzyme and the accumulation of amorpha-4,11-diene ranging from 0.2 to 1.7 ng per g fresh weight.

L7 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:144616 HCAPLUS
DOCUMENT NUMBER: 132:204840
TITLE: Artemisia annua **amorpha-4,11-diene synthase**, its cDNA, recombinant expression, and methods of amorpha-4,11-diene and artemisinin synthesis via

transgenic plants
INVENTOR(S): Wallaart, Thorvald Eelco; Bouwmeester, Hendrik Jan
PATENT ASSIGNEE(S): Neth.
SOURCE: Eur. Pat. Appl., 41 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 982404	A1	20000301	EP 1998-202854	19980827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2340925	AA	20000309	CA 1999-2340925	19990827
WO 2000012725	A2	20000309	WO 1999-EP6302	19990827
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9957423	A1	20000321	AU 1999-57423	19990827
AU 766764	B2	20031023		
EP 1108041	A2	20010620	EP 1999-944535	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 9913196	A	20010925	BR 1999-13196	19990827
JP 2002523101	T2	20020730	JP 2000-567711	19990827
ZA 2001001455	A	20010828	ZA 2001-1455	20010221

PRIORITY APPLN. INFO.: EP 1998-202854 A 19980827
WO 1999-EP6302 W 19990827

AB **Amorpha-4,11-diene**
synthase from Artemisia annua L., its cDNA, recombinant expression, and methods of prepg. amorpha-4,11-diene and artemisinin from farnesyl pyrophosphate (FPP) using transgenic organism are provided. Amorpha-4,11-diene is a precursor of the new anti-malarial drug artemisinin produced by the plant Artemisia annua L. A cDNA encoding **amorpha-4,11-diene synthase** from A. annua has been isolated and sequenced, and the corresponding amino acid sequence has been detd. Recombinant **amorpha-4, 11-diene synthase** expressed in E. coli, transgenic tobacco, and transgenic A. annua catalyzed conversion of FPP into amorpha-4,11-diene. Further conversion of amorpha-4,11-diene into artemisinin was obsd. in transgenic A. annua. The invention may be useful in obtaining enhanced prodn. of stereochem. desirable artemisinin.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2000:826620 HCAPLUS
DOCUMENT NUMBER: 134:189822
TITLE: **Amorpha-4,11-**
diene Synthase of Artemisia annua: cDNA Isolation and Bacterial Expression of a Terpene Synthase Involved in Artemisinin Biosynthesis
AUTHOR(S): Chang, Yung-Jin; Song, Seung-Hwan; Park, Si-Hyung; Kim, Soo-Un
CORPORATE SOURCE: School of Agricultural Biotechnology and the Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon, 441-744, S. Korea

SOURCE: Archives of Biochemistry and Biophysics (2000), 383(2), 178-184
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Artemisia annua, an indigenous plant to Korea, contains an antimalarial sesquiterpene, artemisinin. The first committed step of artemisinin biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene synthase to produce an amorphane-type ring system. The aims of this research were to molecularly clone and express **amorpha-4,11-diene synthase** for metabolic engineering. PCR amplification of genomic **DNA** with a pair of primers, designed from the conserved regions of sesquiterpene synthases of several plants, produced a 184-bp **DNA** fragment. This fragment was used in Northern blot anal. as a probe, showing approx. 2.2 kb of a single band. Its sequence information was used to produce 2106 bp of a full-length cDNA sequence including 1641 bp of open reading frame for 546 amino acids (kcs12) through a rapid amplification of cDNA ends (RACE). The deduced amino acid sequence displayed 36% identity with 5-epi-aristolochene synthase of Nicotiana tabacum. A sol. fraction of Escherichia coli harboring kcs12 catalyzed the cyclization of farnesyl diphosphate to produce a sesquiterpene, which was identified through GC-MS anal. as amorpha-4,11-diene. (c) 2000 Academic Press.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> file registry

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	93.36	93.57

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-8.32	-8.32

FILE 'REGISTRY' ENTERED AT 11:30:40 ON 09 JUN 2004

Property values tagged with IC are from the ZIC/VINITI data file provided by InfoChem.

STRUCTURE FILE UPDATES: 7 JUN 2004 HIGHEST RN 690625-61-7
DICTIONARY FILE UPDATES: 7 JUN 2004 HIGHEST RN 690625-61-7

TSCA INFORMATION NOW CURRENT THROUGH JANUARY 6, 2004

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Crossover limits have been increased. See HELP CROSSOVER for details.

Experimental and calculated property data are now available. For more information enter HELP PROP at an arrow prompt in the file or refer to the file summary sheet on the web at:
http://www.cas.org/ONLINE/DBSS/registryss.html

=> s sesquiterpene cyclase
69 SESQUITERPENE
2788 CYCLASE
L8 10 SESQUITERPENE CYCLASE
(SESQUITERPENE(W)CYCLASE)

```
=> d l8

L8   ANSWER 1 OF 10  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   463369-40-6  REGISTRY
CN   Cyclase, farnesyl pyrophosphate (corn strain McC gene stc1) (9CI)  (CA
     INDEX NAME)
OTHER NAMES:
CN   GenBank AAK73113
CN   GenBank AAK73113 (Translated from: GenBank AF391808)
CN   Sesquiterpene cyclase (corn strain McC chromosome 9 gene stc1)
FS   PROTEIN SEQUENCE
DR   467473-02-5
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS
DT.CA  CAplus document type:  Journal
RL.NP  Roles from non-patents:  BIOL (Biological study); PRP (Properties)

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               2 REFERENCES IN FILE CA (1907 TO DATE)
               2 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> s amorpha-4, 11-diene synthase
            28 AMORPHA
      13759876 4
        873460 11
        210105 DIENE
         25230 SYNTHASE
L9           6 AMORPHA-4, 11-DIENE SYNTHASE
                 (AMORPHA(W)4(W)11(W)DIENE(W)SYNTHASE)

=> d l9

L9   ANSWER 1 OF 6  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   642550-56-9  REGISTRY
CN   DNA (synthetic Saccharomyces cerevisiae amorpha-4,11-diene synthase
     gene) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   37: PN: US20040005678 SEQID: 37 claimed DNA
FS   NUCLEIC ACID SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
DT.CA  CAplus document type:  Patent
RL.P   Roles from patents:  BIOL (Biological study); PRP (Properties); USES
       (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> s amorphadiene synthase
             1 AMORPHADIENE
         25230 SYNTHASE
L10          0 AMORPHADIENE SYNTHASE
                 (AMORPHADIENE(W)SYNTHASE)
```

```
=> d his

     (FILE 'HOME' ENTERED AT 11:18:59 ON 09 JUN 2004)

     FILE 'HCAPLUS, AGRICOLA, BIOSIS, BIOTECHDS' ENTERED AT 11:19:39 ON 09 JUN
     2004
L1            59 S SESQUITERPENE CYCLASE AND DNA
L2            40 DUP REM L1 (19 DUPLICATES REMOVED)
L3            19 S L2 AND 1990-1998/PY
L4            19 FOCUS L3 1-
L5             0 S L4 AND ARTEMISIA ANNUA
L6             9 S (AMORPHA-4,11-DIENE SYNTHASE OR AMORPHADIENE SYNTHASE) AND (D
L7             6 DUP REM L6 (3 DUPLICATES REMOVED)

     FILE 'REGISTRY' ENTERED AT 11:30:40 ON 09 JUN 2004
L8            10 S SESQUITERPENE CYCLASE
L9             6 S AMORPHA-4, 11-DIENE SYNTHASE
L10            0 S AMORPHADIENE SYNTHASE

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    45.09     138.66

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                     0.00      -8.32

STN INTERNATIONAL LOGOFF AT 11:33:12 ON 09 JUN 2004
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FOR IFW

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=> file hcaplus medline biosis agricola scisearch biotechds
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                               0.21       0.21

FILE 'HCAPLUS' ENTERED AT 12:32:16 ON 09 JUN 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'MEDLINE' ENTERED AT 12:32:16 ON 09 JUN 2004

FILE 'BIOSIS' ENTERED AT 12:32:16 ON 09 JUN 2004
COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'AGRICOLA' ENTERED AT 12:32:16 ON 09 JUN 2004

FILE 'SCISEARCH' ENTERED AT 12:32:16 ON 09 JUN 2004
COPYRIGHT 2004 THOMSON ISI

FILE 'BIOTECHDS' ENTERED AT 12:32:16 ON 09 JUN 2004
COPYRIGHT (C) 2004 THOMSON DERWENT AND INSTITUTE FOR SCIENTIFIC INFORMATION

=> s (Amorpha-4,11-diene Synthase or Amorpha-4,11-diene Synthetase)
L1          34 (AMORPHA-4,11-DIENE SYNTHASE OR AMORPHA-4,11-DIENE SYNTHETASE)

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          13 DUP REM L1 (21 DUPLICATES REMOVED)

=> s l2 and dna
L3           3 L2 AND DNA

=> d l3 1-3 ibib ab

L3   ANSWER 1 OF 3  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2003:609986  HCAPLUS
DOCUMENT NUMBER:          139:160786
TITLE:                    Biosynthesis of isopentenyl pyrophosphate using
                          recombinant microbial metabolic pathways
INVENTOR(S):              Keasling, Jay; Martin, Vincent; Pitera, Douglas; Kim,
                          Seon-Won; Withers, Sydnor T.; Yoshikuni, Yasuo;
                          Newman, Jack; Khlebnikov, Artem Valentinovich
PATENT ASSIGNEE(S):       USA
SOURCE:                   U.S. Pat. Appl. Publ., 40 pp.
                          CODEN: USXXCO
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   2
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.   DATE
     --------------    ----  --------       ---------------   --------
     US 2003148479      A1   20030807       US 2001-6909      20011206
     US 2004005678      A1   20040108       US 2003-411066    20030409
PRIORITY APPLN. INFO.:                   US 2001-6909      A2 20011206
AB   Methods for synthesizing isopentenyl pyrophosphate are provided. A first
     method comprises introducing into a host microorganism a plurality of
     heterologous nucleic acid sequences, each coding for a different enzyme in
     the mevalonate pathway for producing isopentenyl pyrophosphate. A related
     method comprises introducing into a host microorganism an intermediate in
     the mevalonate pathway and at least one heterologous nucleic acid
     sequence, each sequence coding for an enzyme in the mevalonate pathway
     necessary for converting the intermediate into isopentenyl pyrophosphate.
     The invention also provides nucleic acid sequences, enzymes, expression
```

vectors, and transformed host cells for carrying out the methods.

L3 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:826620 HCAPLUS
DOCUMENT NUMBER: 134:189822
TITLE: **Amorpha-4,11-diene Synthase** of Artemisia annua: cDNA Isolation and Bacterial Expression of a Terpene Synthase Involved in Artemisinin Biosynthesis
AUTHOR(S): Chang, Yung-Jin; Song, Seung-Hwan; Park, Si-Hyung; Kim, Soo-Un
CORPORATE SOURCE: School of Agricultural Biotechnology and the Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon, 441-744, S. Korea
SOURCE: Archives of Biochemistry and Biophysics (2000), 383(2), 178-184
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Artemisia annua, an indigenous plant to Korea, contains an antimalarial sesquiterpene, artemisinin. The first committed step of artemisinin biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene synthase to produce an amorphane-type ring system. The aims of this research were to molecularly clone and express **amorpha-4,11-diene synthase** for metabolic engineering. PCR amplification of genomic **DNA** with a pair of primers, designed from the conserved regions of sesquiterpene synthases of several plants, produced a 184-bp **DNA** fragment. This fragment was used in Northern blot anal. as a probe, showing approx. 2.2 kb of a single band. Its sequence information was used to produce 2106 bp of a full-length cDNA sequence including 1641 bp of open reading frame for 546 amino acids (kcs12) through a rapid amplification of cDNA ends (RACE). The deduced amino acid sequence displayed 36% identity with 5-epi-aristolochene synthase of Nicotiana tabacum. A sol. fraction of Escherichia coli harboring kcs12 catalyzed the cyclization of farnesyl diphosphate to produce a sesquiterpene, which was identified through GC-MS anal. as amorpha-4,11-diene. (c) 2000 Academic Press.
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:144616 HCAPLUS
DOCUMENT NUMBER: 132:204840
TITLE: Artemisia annua **amorpha-4,11-diene synthase**, its cDNA, recombinant expression, and methods of amorpha-4,11-diene and artemisinin synthesis via transgenic plants
INVENTOR(S): Wallaart, Thorvald Eelco; Bouwmeester, Hendrik Jan
PATENT ASSIGNEE(S): Neth.
SOURCE: Eur. Pat. Appl., 41 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 982404	A1	20000301	EP 1998-202854	19980827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2340925	AA	20000309	CA 1999-2340925	19990827
WO 2000012725	A2	20000309	WO 1999-EP6302	19990827

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        W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU,
            CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
            IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD,
            MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
            SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY,
            KG, KZ, MD, RU, TJ, TM
        RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK,
            ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,
            CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
    AU 9957423        A1   20000321       AU 1999-57423    19990827
    AU 766764         B2   20031023
    EP 1108041        A2   20010620       EP 1999-944535   19990827
        R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
            IE, SI, LT, LV, FI, RO
    BR 9913196        A    20010925       BR 1999-13196    19990827
    JP 2002523101     T2   20020730       JP 2000-567711   19990827
    ZA 2001001455     A    20010828       ZA 2001-1455     20010221
PRIORITY APPLN. INFO.:                   EP 1998-202854   A  19980827
                                         WO 1999-EP6302   W  19990827

AB  Amorpha-4,11-diene
    synthase from Artemisia annua L., its cDNA, recombinant
    expression, and methods of prepg. amorpha-4,11-diene and artemisinin from
    farnesyl pyrophosphate (FPP) using transgenic organism are provided.
    Amorpha-4,11-diene is a precursor of the new anti-malarial drug
    artemisinin produced by the plant Artemisia annua L.  A cDNA encoding
    amorpha-4,11-diene synthase
    from A. annua has been isolated and sequenced, and the corresponding amino
    acid sequence has been detd.  Recombinant amorpha-4,
    11-diene synthase expressed in E. coli,
    transgenic tobacco, and transgenic A. annua catalyzed conversion of FPP
    into amorpha-4,11-diene.  Further conversion of amorpha-4,11-diene into
    artemisinin was obsd. in transgenic A. annua.  The invention may be useful
    in obtaining enhanced prodn. of stereochem. desirable artemisinin.
REFERENCE COUNT:          6     THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> s Amorpha-4,11-diene Synthetase
L4            0 AMORPHA-4,11-DIENE SYNTHETASE

=> s Amorpha-4,11-diene Synth?
   3 FILES SEARCHED...
L5           34 AMORPHA-4,11-DIENE SYNTH?

=> d his

     (FILE 'HOME' ENTERED AT 12:31:40 ON 09 JUN 2004)

     FILE 'HCAPLUS, MEDLINE, BIOSIS, AGRICOLA, SCISEARCH, BIOTECHDS' ENTERED
     AT 12:32:16 ON 09 JUN 2004
L1           34 S (AMORPHA-4,11-DIENE SYNTHASE OR AMORPHA-4,11-DIENE SYNTHETASE
L2           13 DUP REM L1 (21 DUPLICATES REMOVED)
L3            3 S L2 AND DNA
L4            0 S AMORPHA-4,11-DIENE SYNTHETASE
L5           34 S AMORPHA-4,11-DIENE SYNTH?

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    15.84      16.05

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                    -2.08      -2.08
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STN INTERNATIONAL LOGOFF AT 12:34:54 ON 09 JUN 2004